

AMENDMENTS TO THE SPECIFICATION:

Page 1, before line 5, insert the following:

-- This application is a continuation of co-pending Application No. 09/445,205, filed on January 7, 2000. Application No. 09/445,205 is the national phase of PCT International Application No. PCT/FR98/01136 filed on June 4, 1998 under 35 U.S.C. § 371. The entire contents of each of the above-identified applications are hereby incorporated by reference.--

Page 11, replace the paragraph beginning on line 11 as follows:

--The term GFP indicates a protein represented by the sequence SEQ ID NO:2 coded for the nucleotide sequence SEQ ID NO:1 given in Figure 1, and which emits a fluorescence once it is expressed in cells. GFPs with substitutions, additions or deletions of amino acids which have an influence either on the fluorescence properties or on the degree of expression of the GFP are referred to as GFP mutants.--.

Page 34, replace the paragraph beginning on line 21 as follows:

--3) in the receptor sequence, in particular in the first or third intracellular loop, optionally by introducing one or more copies of a spacer sequence, in particular-GGGGS-, represented by the nucleotidic sequence SEQ ID NO:3.--;

Page 34, replace the paragraph beginning on line 25 as follows:

--When the fusion is carried out between EGFP and a receptor-channel (group 2), the fusion can be carried out in particular:

1) in the region homologous to the "major immunogenic region" of the  $\alpha$  sub-unit of the nicotinic receptor of Torpedo (residues 67-76), optionally by introducing one or more copies of a spacer sequence, in particular-GGGGS-, represented by the nucleotidic sequence SEQ ID NO:3.--.

Page 39, replace the paragraph beginning on line 35 as follows:

--- Figure 1 gives the nucleotide sequence SEQ ID NO:1 coding for the wild-type GFP represented by the sequence SEQ ID NO:2 (Prasher et al. 1992, Gene 111:229-233) of *Aequorea victoria*.--.

Page 41, replace the paragraph beginning on line 13 as follows:

--- Figure 6a represents the response functionality test for the release of intracellular calcium (FURA 2) for cells expressing the DNA construct pCEP4-NK2R WT. The fluorescence at 510 nm is given on the y-axis (expressed in counts per second), the excitation taking place at 340 nm, and the time (in seconds) is given on the x-axis. The responses are elicited by the agonist neurokinin A (NKA) and inhibited by the antagonist cyclo(-Gln-Trp-Phe-Gly-Leu-Met) (cyclopeptide) represented by the sequence SEQ ID NO:4. In experiment 1, 10 nM NKA are added. In experiment (2,

3), 5  $\mu$ M of cyclopeptide (2), and then 10 nM of NKA (3) are successively added.--.

Page 45, replace the paragraph beginning on line 24 as follows:

--A restriction site of the endonuclease BsrG I is introduced onto the coding codons 1 to 9 of EGFP using the oligonucleotide 5'GGTCGCCACCCTGTACAAGAAGGGCGAGG3', represented by the nucleotidic sequence SEQ ID NO:5, reagents provided in the mutagenesis kit RPN 1526 (Sculptor) supplied by the Amersham company, and single-stranded pEGFP C3 prepared from the plasmid pEGFP C3 (Genbank Accession No US 57607) supplied by the ClonTech company. The mutant pEGFP C3-1 obtained is sequenced and then cloned in phase with the signal peptide of alpha 7 by ligation of two fragments: the 5225-nt fragment BsrGI-Xho of pJL223 (Eiselé et al. 1993, Nature 366:479-483) and the 725-nt fragment BsrG I-Xho I of pEGFP C3-1. The plasmid pJL223 contains the gene for the protein a7-V201-5HT3 between the sites Not I and Xho I of the vector pMT3 (Swick, A.G. et al. 1992, Proc. Natl. Acad. Sci. 89:1812-1816). The construct obtained, named pMT3-EGFP-C3-SP, is transiently late expressed in HEK 293 cells (ATCC CRL 1573) after transfection with calcium phosphate (Cheng and Okayama 1986), in order to check that the construct is correct. The fluorescent emission spectrum (excitation at 450 nm) of the culture supernatants of cells expressing pMT3-EGFP C3-SP or of and transfected cells (concentrated five-fold by centrifugation on centrikon 10 Amicon)) are recorded. In Figure 2, which shows the difference between the spectrum of transfected and non-transfected cells, the emission peak of EGFP is clearly seen, which indicates

that the construct does indeed lead to the expression of EGFP secreted into the culture medium.--.

Page 46, replace the paragraph beginning on line 34 as follows:

--The single-stranded DNA of pKS NK2R is mutagenized as in 1a) with the oligonucleotides i1: 5'CACGAGAGGATGTACAACCTCGAGCGCACAGTCACC3' represented by the nucleotidic sequence SEQ ID NO:6, containing the mutations for the cloning sites BsrG I and Xho I, allowing the introduction of EGFP between amino acids 65 and 66, and i3: 5'GTACCCAGACACCAGCTAGCAGATCTGAAGCTTCGCCATCAGGC3' represented by the nucleotidic sequence SEQ ID NO:7, containing the mutations for the cloning sites Nhe I, Bgl II and Hind III allowing the introduction of EGFP between residues 233 and 234 or 233 and 238.--.

Page 54, replace the paragraph beginning on line 15 as follows:

--The portion of pCEP4-NK2R-RF1 coding for the fusion protein is amplified by PCR (Current Protocols in Molecular Biology, op.cit.) using the primers 5'GGAGAGTTCCAACCTCGAGAAAAGAAAGAAGGGCGAGGAG3' and 5'GTCAGCTGTTTCTGCGGCGCGCTAAGCCTGGGCCTT3', represented respectively by the nucleotidic sequences SEQ ID NO:8 and SEQ ID NO:9, allowing 1) the production of 1868-nt fragment coding for all of the fusion protein NF2R-EGFP except for the signal peptide, and 2) the in-phase cloning into the expression vector of yeast pPIC9 (Invitrogen) with the sequence coding for the signal peptide of

the promoter factor alpha of the gene AOX1. The cloning sites used are, respectively, XhoI for the 5' end of the amplification product and Not I for its 3' end.--.

Page 55, replace the paragraph beginning on line 5 as follows:

--I) Cloning: the cDNA fragment coding for the human muscarinic receptor M1 (Genbank Accession No X15263) is amplified by PCR (Current Protocols in Molecular Biology, op.cit.) using the primers:

5'TTAGTTCTAAACTAGCGGCCGCACTAGTCCTCCATGAACACTTCAGCCCCA3' and  
5'CTTGAACCTATAGCTAGCCTCGAGTCAGCATTGGCGGGAGGG3', represented  
respectively by the nucleotidic sequences SEQ ID NO: 10 and SEQ ID  
NO:11.;

Page 55 replace the paragraph beginning on line 21 as follows:

--The oligonucleotide 5'CCTGCTGTCTCAGATCTCATCACCGTCC3', represented by the nucleotidic sequence SEQ ID NO:12, is used, together with the reagents in the Sculptor mutagenesis kit (Amersham), to produce a mutant which allows the fusion in position 13 of the sequence coding for the hM1 receptor by means of the introduction of a restriction site for the enzyme Bgl II.--

Page 56, replace the paragraph beginning on line 37 as follows:

--The oligonucleotide  
5'CAGATCATTAGTTGTACAGGAAAGATCTTGAGGATCCTGGAGTGAAG3', represented  
by the nucleotidic sequence SEQ ID NO:13, is used to introduce, on

KS223, the restriction sites for the enzymes BsrG I, Bgl II and BamH I in the same phase as that of the identical sites borne by the plasmid pEGFP-C3. The mutation is introduced into a region of the receptor known as the MIR (Major Immunogenic Region, Barkas et al. 1987, Science, 235:77-80) between amino acids 63 and 64.--.

Page 57, replace the paragraph beginning on line 37 as follows:

--The cDNA coding for human chemokine receptor CCR5 (Genbank access No: U54994) is amplified using the oligonucleotides 5'GGCCCAAGCTTATGTCAGGATCCGGGGAT3', represented by the nucleotidic sequence SEQ ID NO:14, and 5'CGCCCGCTCGAGTCACAAGCCCACAGATAT' represented by the nucleotidic sequence SEQ ID NO:15, and then cloned into the vector Bluescript KS opened with the restriction enzyme Eco RV.--.

Page 58, replace the paragraph beginning on line 31 as follows:

--The cDNA coding for the human chemokine RANTES (Genbank access number M21121) is synthesized by recursive PCR using the oligonucleotides

1            5'GTTGACAAGCTTCGGGATCCA3' represented by the nucleotidic  
sequence SEQ ID NO:16,

2           5' AGCACAGAGGGCAGTAGCAATGAGGATGACAGCG  
               |  
AGGCGTGCCGCGGAGACCTTCATTGGATCCCGAAGCTTGTCAAC3'

represented by the nucleotidic sequence SEQ ID NO:17,

3 5'ATTGCTACTGCCCTCTGTGCTCCTGCATCTGCCTCC  
CCATATTCTCGGACACCACACCATGCTGCTTCGCCTACATT3';

represented by the nucleotidic sequence SEQ ID NO:18,

4           5'GCACTTGCCACTGGTGTAGAAATACTCCTTGATGTGG  
             GCACGGGGCAGTGGGCGGGCAATGTAGGCGAAGCAGCATGG3';  
 represented by the nucleotidic sequence SEQ ID NO:19,

5           5'GCACTTGCCACTGGTGTAGAAATACTCCTTGATGT  
             GGGCACGGGGCAGTGGGCGGGCAATGTAGGCGAAGCAGCATGG3',  
 represented by the nucleotidic sequence SEQ ID NO:20,

6           5'CTAGCTCATCTCCAGCGAGTTGATGTACTCCCGAACC  
             CATTCTTCTCTGGGTTGGCACAACTTGACG3';  
 represented by the nucleotidic sequence SEQ ID NO:21,

7           5'AACTCGCTGGAGATGAGCTAGGCGGCCGCTCG  
             AGGTCGACCTAGTCACTA3', represented by the nucleotidic  
 sequence SEQ ID NO:22,

8           5'TAGTGACTAGGTCGACCTCGA3',       represented       by       the  
 nucleotidic sequence SEQ ID NO:23, according to the protocols  
 described in Prodromou & Pearl, Protein Engineering Vol 5, pp 827-  
 829, 1992, and then cloned into the vector Bluescript KS opened  
 with Eco RV.--.

Page 60, replace the paragraph beginning on line 15 as  
 follows:

--The cDNA coding for the alpha sub-unit of the mouse  
 protein Gq (Genbank Accession No M55412) is amplified by PCR using  
 the oligonucleotides 5'GCGGCCGCATGGGGGATCCTACTCTGGAGTCCATCATGGCG  
 and  
 5'CCGCTCGAGTTAATCTAGAAGGACCAGATTGTACTCCTTCAGG,       represented  
 respectively by the nucleotidic sequences SEQ ID NO:24 and SEQ ID  
 NO:25, in order to introduce Not I and Bam HI sites at the 5' end  
 and Xho I and Xba I at the 3' end of the gene coding for the alpha  
 sub-unit of the protein Gq.--.